Atty. Docket No.:   4231/2042   **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Choong-Chin Liew	Examiner:	Jennifer Ann Dunston
Serial No.:	10/661,242		
Filed:	September 12, 2003	Group Art Unit:	1636
Titled:	IDENTIFICATION OF SEQUENCES PARTICULARLY USEFUL FOR THE DIAGNOSIS AND IDENTIFICATION OF THERAPEUTIC TARGETS FOR OSTEOARTHRITIS	Conf. No.:	9495

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF Hongwei Zhang UNDER 37 C.F.R. §1.132

Sir:

    I, Hongwei Zhang, Ph.D., hereby declare that:

1.   I received a Ph.D. degree from the Institute of Medical Science at the University of Toronto in 2002, and a Master of Science degree from the Department of Immunology at the University of Toronto in 1995. In addition I received my Medical Degree from the University of Medical Sciences in Changchun China in 1989 and practiced as a staff physician for 4 years in Beijing prior to commencing my post graduate studies. I currently hold the positions of Senior Scientist and Scientific Program Leader of Functional Genomics as well as Manager of Research and Development at ChondroGene Inc.

    I am one of the inventors of the above-noted U.S. patent application.

    I am particularly experienced in the field of osteoarthritis having worked as a Research Associate at the Arthritis Center of Excellence of

Toronto Western Hospital, and subsequently receiving a Fellowship from the institute to pursue my Ph.D. studies focusing on the area of osteoarthritis. Subsequently I have been one of the key scientists involved in the ongoing collaboration of GeneNews (formerly ChondroGene) with Pfizer in the area of osteoarthritis and biomarker discovery. I am a trained molecular biologist experienced in developing methods to identify biomarkers which are indicative of a disease or condition, and in developing methods of using these biomarkers and products thereof as applied in the area of osteoarthritis, amongst other conditions.

List of Publications:

K.W. Marshall, M.D., PhD., F.R.C.S., **H. Zhang, M.D., PhD.**, T.D. Yager Ph.D., N. Nossova M.D., Ph.D., A. Dempsey PhD., R. Zheng M.D., M. Han M.D. Ph.D., H.Tang M.Sc., S. Chao M.A.Sc, and C.C. Liew PhD. "Blood-based biomarkers for detecting mild osteoarthritis in the human knee" *OsteoArthritis and Cartilage (2005)* 861-871.

Zhang H, Marshall KW, Tang H, Hwang DM, Lee M, Liew CC. Profiling genes expressed in human fetal cartilage using 13,155 expressed sequence tags. Osteoarthritis Cartilage 2003;11:309-19.

Hongwei Zhang, C.C.Liew, K.Wayne Marshall. Microarray Analysis Reveals the Involvement of Beta-2 Microglobulin (B2M) in Human Osteoarthritis. Osteoarthritis and Cartilage 2002;10:950-60.

Doherty PJ, **Zhang H**, Manolopoulos V, Trogadis J, Tremblay L, Marshall KW. Adhesion of transplanted chondrocytes onto cartilage in vitro and in vivo. J Rheumatol 2000;27:1725-312.

Zhao YX, Lajoie G, **Zhang H,** Chiu B, Payne U, Inman RD. Tumor necrosis factor receptor p55-deficient mice respond to acute Yersinia enterocolitica infection with less apoptosis and more effective host resistance. Infect Immun 2000;68:1243-513.

Vaselios Manolopoulos, K. Wayne Marshall, **Hongwei Zhang**, Judy Trogadis, Louise Trembly and Paul J. Doherty. Factors affecting the efficacy of bovine chondrocyte transplantation in vitro. Osteoarthritis and Cartilage 1999;7:453-460.

Yi-Xue Zhao, **Hongwei Zhang**, Basil Chiu, Usulra Payne, Robert D. Inman. Tumor necrosis factor receptor P55 controls the severity of arthritis in

experimental *Yersinia Enterocolitica* infection.   Arthritis & Rheumatism 1999;42:1662-1672.

Paul J. Doherty, **Hongwei Zhang**, Louise Trembley, Vaselios Manolopoulos and K. Wayne Marshall. Resurfacing of articular cartilage explants with genetically-modified human chondrocytes *in vitro*. Osteoarthritis and Cartilage 1998;6:153-160.

Hongwei Zhang, Donna Phang, Ronald M. Laxer, Earl D. Silverman, Sueihua Pan, and Paul J. Doherty. Evolution of the T cell receptor beta repertoire from synovial fluid T cells of patient with juvenile onset rheumatoid arthritis. J. Rheumatol. 1997;24:1396-402.

Petro Lastres, Anihoa Letamendia, **Hongwei Zhang**, Carlos Rius, Nuria Almendro, Ulla RAab, Louis A. Lopez, Carmen Langa, Angels Fabra, Michelle Letarte and Carmelo Bernabeu. Endoglin modulates cellular responses to TGF-beta 1. J. Cell Biol. 1996;133:1109-1121.

Hongwei Zhang, Andrew R.E. Shaw, Allan Mak, and Michelle Letarte. Endoglin is a component of the Transforming Growth Factor (TGF)-beta receptor complex of human pre-B leukemic cells. J. Immunol. 1996,156:565-573.

2.   I have read the Office Action mailed July 26, 2006 in the above-referenced patent application.

The Office Action states that claims 58-73 are rejected under 112 first paragraph, enablement.

The independent claims being instantly filed in response to the office action are currently amended claims 37, 38, 42, 46 and 50. Currently amended claim 37 is drawn to diagnosis of mild OA by comparison of expression of RNA encoded by a gene, for each the genes TNFAIP6 and TGFBI, between a test individual and normal control individuals.

I understand that concerns which were raised by the Examiner and which are addressed in this Declaration with respect to the instantly filed claims relate to the numbers of samples used in microarray hybridization assays, expression fold-change of differentially expressed RNA, statistical significance of

disclosed differential RNA expression, and the specificity and sensitivity of biomarker differential expression used for diagnosis.

3.   As a scientist skilled in the area of osteoarthritis and molecular biomarker identification, I submit that the specification provides guidance for diagnosing mild OA by determining differential TNFAIP6 and TGFBI gene expression levels in cartilage between a test individual and normal control individuals.

In our work described in the patent application, we report experiments identifying genes having differential RNA expression in cartilage of individuals having OA which were performed using a ChondroChip microarray (Examples 5-6, Figures 1-6). I hereby submit that these experiments were performed using samples from 6 individuals diagnosed as not having OA ("normal" control), 3 individuals diagnosed as having mild OA, 8 individuals diagnosed as having moderate OA, 7 individuals diagnosed as having mild OA marked OA, and 13 individuals diagnosed as having severe OA. In the patent application we further report experiments identifying genes having differential RNA expression which were performed using Affymetrix U133A microarray (Example 6, Figure 7). I thereby submit that these experiments were performed using samples from 10 individuals diagnosed as not having OA ("normal" control), 4 individuals diagnosed as having mild OA, and 5 individuals diagnosed as having severe OA.

I submit that the experiments disclosed in the specification demonstrate that TNFAIP6 and TGFBI express RNA at differential levels between individuals having mild OA and normal control individuals so as to enable diagnosis of mild OA in accordance with currently amended claim 37. As described below, subsequent to filing of the application we have performed additional experiments, using samples representing an expanded and non-overlapping set of individuals having mild OA relative to those used in the specification, which confirm that the method of currently amended claim 37 is enabled.

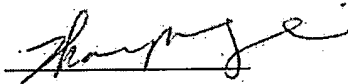## TGFBI and TNFAIP6 RNA Expression is Significantly Up-regulated in Mild OA Cartilage vs Normal Cartilage

Attached as Exhibit "A" to this Declaration is a summary and analysis of post-filing experimental data from experiments which we, the inventors, have obtained relating to RNA expression levels of TNFAIP6 and TGFBI in human subjects diagnosed as having mild OA or as not having OA ("Normal"). The data represent TNFAIP6- or TGFBI-specific fluorescent hybridization signal intensities obtained using Affymetrix U133A microarray analysis of cDNA derived from cartilage samples from 10 normal individuals (i.e. diagnosed as not having OA), and from 20 individuals diagnosed as having mild OA. The mean fold-changes of cDNA levels, which represent RNA expression levels, specific to TGFBI and TNFAIP6 in mild OA versus normal samples were found to be 5.2-fold and 6.1-fold, respectively, signifying that RNA expression of both genes is upregulated on average in mild OA cartilage relative to normal cartilage. Using MEDCALC software, the signal intensity data for each gene was analyzed via the ROC curve approach to determine the optimal expression level threshold to differentiate between RNA expression levels of the genes in mild OA cartilage and normal cartilage. Expression level threshold values of 0.531 and 0.4 were determined for TGFBI and TNFAIP6, respectively, and each sample was given a score assigning it as being sub-threshold ("0") or supra-threshold ("1"). As can be seen, for TGFBI, 8/10 normal samples scored below threshold, and 19/20 mild OA samples scored above threshold (p < 3.18E-6). Similarly for TNFAIP6, 9/10 samples scored below threshold and 19/20 mild OA samples scored above threshold (p < 8.17E-7). Thus, RNA expression of both genes exhibits consistent up-regulation in mild OA cartilage relative to normal cartilage. Samples were then assigned a classifier value characterizing them as "mild OA", or "normal" depending on whether RNA expression of neither gene, or of both genes, respectively, was upregulated in mild OA relative to normal cartilage. As can be seen, 7/10 normal samples were correctly classified as "normal", while 18/10 mild OA samples were correctly classified as "mild OA". These results hence signify that levels of

TGFBI and TNFAIP6 in cartilage can be used to determine an indication of mild OA in a human test subject with a specificity of 90% and a sensitivity of 70%.

In view of the above, I submit that the specification enables one of skill in the art to practice the method of currently amended claim 37.

5. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that wilful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Hongwei Zhang, Ph.D. _____

Date _January 26, 2007_

EXHIBIT "A"

			Fluorescence threshold		
			TGFBI	TNFAIP6	
			0.531	0.4	
Sample ID	Fluorescence intensity (relative mRNA level)		Score (0: ≤ threshold; 1: > threshold)		Classifier Score (0: "normal"; 1: "mild OA" 1/0: equivocal)
	TGFBI	TNFAIP6	TGFBI	TNFAIP6	
Normal, ID C103	0.0523	0.238	0	0	0
Normal, ID C104	0.514	0.0783	0	0	0
Normal, ID C106	0.111	0.246	0	0	0
Normal, ID C108	0.531	0.363	0	0	0
Normal, ID C110	0.354	0.209	0	0	0
Normal, ID C111	0.685	0.165	1	0	1/0
Normal, ID C114	0.102	1	0	1	1/0
Normal, ID C115	0.15	0.257	0	0	0
Normal, ID C120	0.726	0.214	1	0	1/0
Normal, ID C121	0.337	0.247	0	0	0
Mild OA, ID 276	1.038	3.357	1	1	1
Mild OA, ID 322	2.217	3.402	1	1	1
Mild OA, ID 335	0.711	0.541	1	1	1
Mild OA, ID 342	2.762	1.596	1	1	1
Mild OA, ID 365	1.871	1.101	1	1	1
Mild OA, ID 407	2.799	0.72	1	1	1
Mild OA, ID 451	3.151	0.933	1	1	1
Mild OA, ID 460	2.344	0.742	1	1	1
Mild OA, ID 465	1	3.325	1	1	1
Mild OA, ID 515	0.561	0.798	1	1	1
Mild OA, ID 544	2.161	1.157	1	1	1
Mild OA, ID 584	2.711	0.801	1	1	1
Mild OA, ID 673	5.066	2.494	1	1	1
Mild OA, ID 704	0.712	1.084	1	1	1
Mild OA, ID 798B	1.726	2.299	1	1	1
Mild OA, ID 812	2.646	4.887	1	1	1
Mild OA, ID 824	0.606	2.711	1	1	1
Mild OA, ID 848B	0.333	1.611	0	1	1/0
Mild OA, ID 908A	0.912	1.653	1	1	1
Mild OA, ID 944B	1.368	0.318	1	0	1/0
	Summary	Mild OA	19/20	19/20	18/20
		Control	8/10	9/10	7/10
			TGFBI	TNFAIP6	TNFAIP6 & TGFBI
		True negatives	8	9	8
		True positives	19	22	19
		Equivocal			5
		Accuracy	90%	93%	83%
		Specificity	95%	95%	90%
		Sensitivity	80%	90%	70%
		Fold-change (mild OA/normal)	5.2	6.1	
		p-value	< 3.18E-6	< 8.17E-7	